

IN THE CLAIMS

Claim 1 (currently amended): A method of analysing a plurality of biological entities using an imaging apparatus, the method comprising:

- a) acquiring a first image of the biological entities;
- a) ~~providing a marker for b) adding a marker to said plurality of biological entities after the first image is acquired,~~ said marker being capable of identifying objects within said plurality of biological entities when detected using the imaging apparatus, ~~wherein said marker is capable of identifying said objects during a first time period, and said marker is less capable of identifying said objects during a second time period;~~
- b) ~~during the first time period, c) recording a marked-up image in which spatial definitions of said objects are identifiable from said marker; and~~
- e) ~~during the second time period, recording a first image of said plurality of biological entities; and~~
- d) generating a spatial definition for an object in said first image using data derived from said marked-up image.

Claim 2 (currently amended): The method of claim 1, ~~wherein further comprising acquiring an initial series of images before adding a markerthe marked up image and recording a marked up image, and applying the spatial definition to the initial series of images to enable an operator to evaluate changes in the object over timesaid first time period is previous to said second time period.~~

Claim 3 (canceled)

Claim 4 (canceled)

Claim 5 (previously presented): The method of claim 1, wherein said marker has a temporally-varying signal.

Claim 6 (previously presented): The method of claim 5, wherein said marker is provided by a genetic construct system.

Claim 7 (previously presented): The method of claim 1, wherein said generated spatial definition includes at least one of a spatial extent and locational data of the object.

Claim 8 (previously presented): The method of claim 1, wherein the generated spatial definition is generated using a spatial definition of the object detected from said marked-up image.

Claim 9 (currently amended): The method of claim 1, further comprising:

e) ~~during the first time period, recording a further image concurrently with the marked-up image of said plurality of biological entities; and~~

f) deriving spatial definition data from said further image, and ~~in step d), analysing said first image using the data derived from the further image.~~

Claim 10 (previously presented): The method of claim 9, wherein said further image is recorded in a first colour channel and said marked-up image is recorded in a second, different colour channel.

Claim 11 (previously presented): The method of claim 10, wherein said first image is recorded in said first colour channel.

Claim 12 (previously presented): The method of claim 9, further comprising, in step f) deriving data from said further image using data derived from said marked-up image.

Claim 13 (previously presented): The method of claim 9, wherein the data derived in step f) comprises a value or values of one or more characteristics associated with the object.

Claim 14 (previously presented): The method of claim 13, wherein the one or more characteristics include at least one selected from the group consisting of a mean intensity, a standard deviation, a variance, a kurtosis, an autocorrelation function, a spatial correlation

measure, a textual correlation measure, an auto correlation function, a fractal dimension, an area, a perimeter, a length of a principle axis, a width of a principle axis, a compactness and an orientation.

Claim 15 (previously presented): The method of claim 1, wherein step d) further comprises:

- i) defining one of a plurality of test spatial definitions;
- ii) calculating a value of one or more characteristics of the first image using the test spatial definition;
- iii) repeating steps i)-ii) for a different one of the plurality of test spatial definitions;
- iv) selecting one of the plurality of test spatial definitions according to the value or values calculated in step ii).

Claim 16 (previously presented): The method of claim 15, wherein step iv) further comprises comparing said value calculated in step ii) with a value derived from said further image in step f).

Claim 17 (previously presented): The method of claim 16, wherein said comparing comprises calculating a Euclidean distance  $E$ , said Euclidean distance  $E$  being calculated by the following relation:

$$E = \sqrt{\sum_{i=1}^K (Z_N[i] - Z_{N-1}[i])^2}$$

wherein both the value calculated in step ii) and the value derived from said further image in step f) are vectors, respectively  $Z_{N-1}$  and  $Z_N$ , relating to an integer number  $K$  of characteristics  $i$ .

Claim 18 (previously presented): The method of claim 17, wherein step iv) comprises

selecting a substantially minimised value of the Euclidean distance  $E$ .

Claim 19 (previously presented): The method of claim 16, wherein said comparing comprises calculating at least one of a cityblock function, a chebyshev distance, a minkowski of order m function, a quadratic function, a Q-positive definite function, a Canberra distance, a non-near distance function, or an angular separation.

Claim 20 (previously presented): The method of claim 1, further comprising repeating step d) to generate a plurality of spatial definitions for a plurality of objects in said first image.

Claim 21 (previously presented): The method of claim 20, wherein the plurality of generated spatial definitions are filtered according to a quality criterion.

Claim 22 (previously presented): The method of claim 20, wherein step d) further comprises determining a surrounding space of an object detected from said marked-up image, said surrounding space having a boundary separating the surrounding space from at least one different surrounding space of a proximate, different, object and arranging the generated spatial definition to be within the determined surrounding space of the object.

Claim 23 (previously presented): The method of claim 22, comprising determining the surrounding space of the object using a Voronoi algorithm.

Claim 24 (previously presented): The method of claim 1, further comprising recording a second image of the plurality of biological entities during a third time period and generating a spatial definition for an object in said second image.

Claim 25 (previously presented): The method of claim 1, wherein said biological entities are biological cells or cellular components.

Claim 26 (previously presented): The method of claim 25, wherein said objects comprise biological cell nuclei.

Claim 27 (previously presented): The method of claim 25, wherein said objects comprise biological cell mitochondria, biological cell cytoplasm, biological cell lysosomes or bound

antibodies.

Claim 28 (previously presented): The method of claim 25, wherein said objects include at least two selected from the group consisting of biological cell nuclei, biological cell mitochondria, biological cell lysosomes, biological cell cytoplasm and bound antibodies.

Claim 29 (previously presented): The method of claim 28, wherein when said objects include a bound antibody and said biological cells are fixed prior to said providing of the marker.

Claim 30 (previously presented): The method of claim 1, further comprising providing a second, different, marker for said plurality of biological entities, said second marker being additionally used to generate a spatial definition for an object in said first image.

Claim 31 (previously presented): The method of claim 30, wherein said second marker is one of a biological cell dye, a biological cell mitochondria dye, a biological cell lysosome dye or a biological cell cytoplasm dye.

Claim 32 (previously presented): The method of claim 1, further comprising analysing characteristics of the plurality of biological entities by analysing said first image using said generated spatial definition.

Claim 33 (currently amended): A method of image analysis for analysing a plurality of biological entities from images produced using an imaging apparatus, the method comprising:

- a) obtaining a marked-up image of said plurality of biological entities, said marked-up image ~~having been recorded during a first time period in which a marker provided for said plurality of biological entities is being~~ capable of identifying objects within said plurality of entities;
- b) obtaining a first image of said plurality of biological entities, said first image ~~having been recorded during a second time period in which said marker is less capable of identifying said objects being acquired prior to an introduction of a marker;~~ and
- c) generating a spatial definition of an object for said first image using data derived

from said marked-up image.

Claim 34 (currently amended): ~~Computer software arranged to perform the method of claim 1~~ A non-transitory computer readable medium for programmed to instruct a computer to:

- a) acquire a first image of the biological entities, the first image being acquired prior to an introduction of a marker;
- b) add the marker to said plurality of biological entities, said marker being capable of identifying objects within said plurality of biological entities when detected using the imaging apparatus;
- c) record a marked-up image in which spatial definitions of said objects are identifiable from said marker; and
- d) generate a spatial definition for an object in said first image using data derived from said marked-up image.

Claim 35 (canceled)

Claim 36 (canceled)

Claim 37 (new) A imaging apparatus for analysing a plurality of biological entities, said imaging apparatus comprising a computer programmed to:

acquire an initial series of images of the biological entities, the initial series of images being acquired prior to an introduction of a marker;

record a marked-up image, after a marker is introduced to the biological entities, in which spatial definitions of said objects are identifiable from said marker;

generate a spatial definition for an object in the initial series of images using data derived from said marked-up image; and

applying the spatial definition to the initial series of images to enable an operator to evaluate changes in the object over time.